IMPROVED L.E. CELL TEST USING FINGER-PRICK BLOOD

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The L.E. cell is recognized as a neutrophil polymorphonuclear leucocyte whose nuclear lobes are compressed to the periphery of the cell by the inclusion of homogeneous material which stains a reddish purple with the usual blood stains. There is much evidence in favour of the inclusion material being nuclear in origin. Occasionally cells are found in which an ingested cell can still be recognized; these cells are "tart" cells and must not be confused with L.E. cells, in which the ingested material has lost all its nuclear structure.

The production of L.E. cells from bone marrow or blood of patients suffering from systemic lupus erythematosus (S.L.E.) would appear to be essential for a complete diagnosis. Many methods have been devised for producing L.E. cells from venous blood, amongst which the one devised by Marten and Blackburn (1953) and used by us for some time has been found reliable. It often happens that L.E. cells are detected only after several specimens of blood have been examined, and these repeated venepunctures become very tiresome for patient and physician. The processing of venous blood specimens and the microscopical examination of the final blood smears are also time-consuming.

A method in which capillary blood could be used would solve some of these problems, and in fact L.E. cells have been found in finger-prick blood preparations by several workers (Eppes and Ludovic, 1951; Rohn and Bond, 1952; Chomet *et al.*, 1953; Rosenfeld *et al.*, 1954; Sickley *et al.*, 1955); but these have been chance findings or have involved techniques which could not be adapted for routine use.

Snapper and Nathan (1955a, 1955b) have devised a more practical method which requires only one drop of blood from an S.L.E. patient, and it is based on the recognition that three essential factors are involved in L.E. cell formation: (1) plasma or serum from an S.L.E. patient; (2) nucleoprotein from nuclei of damaged cells, which form the "substrate"; and (3) active phagocytic polymorphonuclears. They recommend that the substrate be prepared from a drop of normal blood incubated for an hour in a moist-chamber at 37° C. The blood clot which forms is removed, and it is then found that large numbers of leucocytes, particularly polymorphonuclears, are left on the air-dried slide. The test proper is then carried out by making a hanging-drop preparation of the patient's blood so that the drop of blood just touches the substrate on the slide. This hanging-drop preparation is then incubated in a moist-chamber at 37° C. for one hour. During this period the L.E. factor in the patient's serum acts on the nucleoprotein of the dead leucocytes in the substrate to form an

intermediate product. The latter undergoes phagocytosis by the live polymorphonuclears migrating from the small drop of blood which clots. After incubation the clot is removed and the material on the slide stained with the usual blood stains. The small area on the slide is quickly identified and easily examined microscopically in a very short time.

In our hands the use of leucocytes from normal blood, which is strongly recommended by these workers, has not proved as reliable as a substrate which we describe below prepared from chronic lymphatic leukaemia blood. Details of this method are given and the results, with illustrations, discussed.

Methods

Preparation of Substrate Suspension.—Fifteen millilitres of venous blood from a chronic lymphatic leukaemia patient (white-cell count over 75,000 per c.mm.) is placed in a sterile 1-oz. (28-ml.) screw-capped bottle containing two partially opened steel-wire paper-clips. The bottle is slowly inverted intermittently until the formation of fibrin occurs, and then shaken rapidly for another five minutes. Complete defibrination occurs within 15 minutes of taking the blood, the fibrin becoming attached to the paper-clips. The blood is decanted into a sterile 6 by $\frac{1}{2}$ in. (15 by 1.3 cm.) test-tube, plugged or covered and then inclined at an angle of 60 degrees in a 37° C. water-bath until the red blood cells have sedimented about one-third of the way down. This usually takes one hour, but may take up to three hours. The opalescent supernatant and a buffy layer containing the bulk of the lymphocytes and a small proportion of red blood cells (about 5%) are pipetted off into a 3 by $\frac{1}{2}$ in. (7.5 by 1.3 cm.) test-tube. The cell suspension is mixed gently and a white-cell count carried out. If the white-cell count is below 200,000 per c.mm. the suspension is centrifuged for five minutes at 2,500 r.p.m. and then some of the supernatant removed to give the correct cell count of 200,000 per c.mm. If the count is much above 200,000 per c.mm. dilution can be made with the patient's serum, obtained by centrifuging the blood remaining in the 6 by $\frac{1}{2}$ in. (15 by 1.3 cm.) test-tube.

Preparation of Substrate Slide.—New 3 by 1 in. (7.5 by 2.5 cm.) microscope slides are used, rinsed in hot water and wiped dry. A small drop of the substrate suspension, about 1 to 2 mm. in diameter, is placed at the centre of the slide. With a narrow smooth-ended glass rod the drop is spread out to cover a circular area 12 to 15 mm. in diameter, which takes only a short time to dry at room temperature.

Storage of Substrate Slides.—A large stock of many hundreds of dried substrate slides prepared in the above manner are stored in closely packed cardboard boxes in a laboratory bench drawer at room temperature. The temperature varies between 17 and 20° C. Storage in the refrigerator or at temperatures much above the usual room temperatures causes deterioration.

Finger-prick Blood Technique.—A $\frac{3}{4}$ in. (2.2 cm.) square cover-glass (No. 2 thickness) is divided in half. The two pieces are positioned on opposite sides of the dried substrate area of a substrate slide placed in a Petri dish, the bottom of which contains a well-moistened perfectly flat filter-paper. A drop of blood from a finger-prick is allowed to touch the centre of a 1 $\frac{1}{4}$ by $\frac{3}{4}$ in. (3.2 by 2.2 cm.) cover-glass (No. 1 thickness) so that a drop of blood 4 mm. in diameter is obtained. This cover-glass is now lowered on to the slide and the drop of blood positioned on the periphery of the substrate area. The drop of blood on the No. 1 cover-glass just touches the substrate because of the pieces of No. 2 cover-glass, which are thick enough to prevent obliteration of the drop by capillary action. The Petri dish is now covered with its lid and transferred to a 37° C. incubator for one hour only. The slide with its cover-glass preparation is then gently removed from the Petri dish and any moisture on the under side of the slide wiped off. The large cover-

glass is now slid off. If the clot sticks to the slide it can easily be released with the corner of the cover-glass and then slid to the edge of the slide and removed. The slide is rapidly dried by waving it in the air. Our preparations are stained as for blood with Jenner-Giemsa stains.

Test for L.E. Factor in Serum.—A drop of the serum is placed in a small watch-glass. Two drops of blood from a healthy group O subject are added and quickly mixed. A

loopful of the mixture is placed in the centre of a 1½ by ¾ in. (3.2 by 2.2 cm.) cover-glass and inverted over a substrate slide and further processed as described in the finger-prick technique above.

Examination of Preparations.

—The preparations, after staining, will show a blue area due to the lymphocytes in the substrate. There is a relatively clear or pinkish area where the polymorphonuclears have migrated and red blood cells have been deposited from the drop of blood. This latter area is where L.E. cells, if present, will be found. The whole of the stained area is lightly smeared with immersion oil and examined with low-power and then high-power or oil-immersion objectives. The appearances are as in Figs. 1, 2, and 3 respectively.

Comparison of Various Substrates

Substrates were prepared from normal blood as described by Snapper and Nathan (1955a, 1955b) and also from chronic lymphatic leukaemia, chronic myeloid leukaemia, and various acute leukaemia bloods prepared as described in this paper.

The substrates were tested with finger-prick blood from patients who were suffering from S.L.E. and who showed L.E. cells in venous blood preparations made by the Marten and Blackburn (1953) method, henceforth referred to as the venous blood method. The results were as follows:

Normal Blood Leucocytes Substrate.—Variable results were obtained even with substrate preparations less than 10 days old. In several cases no L.E. cells could be found with the finger-prick blood method although moderate numbers were being found by the venous blood method.

Chronic Myeloid and Acute Leukaemia Substrate.—False-negative results were obtained as with normal blood leuco-

cytes substrate. There was also difficulty in assessing which was the reaction area and which the substrate area.

Chronic Lymphatic Leukaemia Substrate.—L.E. cells were found with the finger-prick blood method in every case where they had been found by the venous blood method. In some cases a more conclusive picture was obtained with this substrate and finger-prick blood than by the venous blood method. Substrates up to three months old still gave reliable results.

Results of the Finger-prick L.E. Test

Normal Subjects.—Of 50 normal subjects, 75% showed an occasional "tart" cell. In two normal subjects an occasional cell morphologically indistinguishable from the L.E. cell was found. The cells were not always found on repeating the tests with a substrate slide made either from the same or from different lymphatic leukaemia blood. This was quite different from the cases of S.L.E., where L.E. cells in moderate or large numbers were consistently found on repeated examinations. In the Table we have denoted this as "positive."

Results of L.E. Cell Tests in 10 Patients who were Clinically Cases of Systemic Lupus Erythematosus

Patient	Age	Sex	L.E. Cell Test	
			Venous Blood	Finger-prick Blood
1	51	M	Positive	Positive
2	64	M	Negative*	"
3	46	M	Positive	"
4	42	F	Not done†	"
5	30	F	Positive	"
6	41	F	"	"
7	11	M	"	"
8	21	M	"	"
9	24	F	"	"
10	41	F	"	"

* Only tart cells found. † Patient refused venepuncture.

Systemic Lupus Erythematosus.—A total of 10 "positive" cases have been seen in the past year. Eight of these showed numerous L.E. cells by the finger-prick and venous blood methods. The other two showed moderate numbers of L.E. cells only by the finger-prick method (see Table). One of these latter patients had refused a venepuncture. Following treatment with cortisone the patients have all improved clinically and the number of L.E. cells in finger-prick and venous blood preparations has diminished.

Patients Suffering from Other Diseases.—Scanty L.E. cells have been found in only one or two cases of rheumatoid arthritis, but were not found consistently on repeat examinations.

Discussion

Harvey *et al.* (1954) have noted that in systemic lupus erythematosus L.E. cells are not found constantly, and repeated testing is necessary. L.E. cells were originally found in bone-marrow preparations (Hargraves *et al.*, 1948), but venous blood preparations were later shown to give reliable results (Hargraves, 1949; Sundberg and Lick, 1949).

Eppes and Ludovic (1951) found only one L.E. cell in peripheral blood smears taken from numerous patients, and Chomet *et al.* (1953) found L.E. cells in the peripheral blood smears of a patient three days before death. They put forward the theory that the L.E. factor was then at its peak concentration, but were uncertain that the L.E. cell was an *in vivo* phenomenon, since a peripheral blood smear could be regarded as a post-vital artefact. Rohn and Bond (1952) actually observed L.E. cell formation in a cover-glass preparation of a drop of blood stained with supravital stains. This method, while of great interest from the point of view of L.E. cell formation, would appear to be unsuitable for routine use. From their observations they concluded that the polymorphonuclears and not lymphocytes underwent autolysis, and the product was then ingested by active polymorphonuclears to produce L.E. cells. This is an opposite

FIG. 1.—Low-power appearance of a positive stained preparation. (×300.)

FIG. 2.—High-power appearance. (×500.)

FIG. 3.—Appearance with oil-immersion objective. (×950.)

view to the one put forward by Moyer and Fisher (1950), who gave experimental evidence that the lymphocyte was the autolysed cell involved and that the best results were obtained with lymphocytes whose cell walls were first damaged. Moyer and Fisher concluded that three factors were necessary for L.E. cell formation: (1) L.E. plasma or serum factor; (2) nucleoprotein from damaged lymphocytes (substrate); and (3) active neutrophils.

Zimmer and Hargraves (1952) compared various methods for producing L.E. cells and decided that methods in which clotted blood was used gave the best results. The method of Marten and Blackburn (1953) is such a method; we have used it for the past two years and found it satisfactory.

Thus it became feasible that the peripheral or finger-prick clotted-blood methods might be developed if factors 2 and 3 previously mentioned could be obtained in an adequate concentration. Snapper and Nathan (1955a, 1955b) have devised an ingenious method for doing this. The nucleoprotein factor, or substrate, is obtained by incubating a drop of blood for an hour at 37° C. in a moist-chamber. An outpouring of neutrophils occurs, and these cells remain on the slide after the blood clot is removed. The air-dried neutrophils act as substrate. A drop of the patient's blood is now incubated in the same area as the substrate. If the patient is suffering from S.L.E., then the L.E. factor in the serum causes an autolysis of the dead neutrophils and the product is phagocytosed by the fresh neutrophils which migrate from the patient's blood clot. Snapper and Nathan mention that cells other than neutrophils can be used as substrate—for example, leukaemic blood cells or tissue cells from Hodgkin's disease or carcinoma.

We have confirmed these authors' observations but find their method for producing substrate slides rather difficult. The preparations were stable for only 10 days, and we have not been able to find L.E. cells by their method when undoubtedly positive results were found by the venous blood method. However, with the use of lymphocytes from chronic lymphatic leukaemia, which are regularly available in a large teaching hospital, much more reliable substrate slides, which were stable for several months, were obtained. The results compared well with the venous blood method. The fact that lymphocytes give such good results would seem to confirm the conclusions of Moyer and Fisher (1950) concerning the mechanism of L.E. cell formation.

The high proportion of false-negative results obtained when leucocytes from normal blood are used as substrate may be due to the small proportion of lymphocytes that are to be found in the substrate area. Polymorphonuclears will always be found there, and one is not certain whether they were derived from the patient's drop of blood. When lymphocytes from blood of patients with chronic lymphatic leukaemia are used as substrate there is little doubt about the source of the polymorphonuclears seen. There appears to be an optimum concentration of lymphocytes required for the test, and to achieve this we recommend placing the patient's drop of blood on to the periphery of the lymphocyte substrate area. The test should always be done in duplicate or triplicate.

Substrates prepared from the buffy coats of normal blood and leukaemias other than chronic lymphatic leukaemia have often given poor results. Kurnick *et al.* (1953) have discussed the probability of an L.E. cell phenomenon inhibitor, and it is possible we are dealing with such an inhibitor when failures occur. It is worth noting that the polymorphonuclear is the cell which can produce this inhibition.

Recently a method has been reported for producing L.E. cells from finger blood in which the digit is first constricted for 20 minutes and tight enough to produce cyanosis (Sickley *et al.*, 1955). This procedure appeared to be rather upsetting to the patient and not reliable, as a high proportion of false negatives were obtained by these workers. Since in S.L.E. Raynaud's phenomenon can occur, the procedure might lead to gangrene.

Fifteen millilitres of blood from one patient with chronic lymphatic leukaemia provides many hundreds of substrate slides, which can be stored for future use by a group of hospitals. The slides are stable at room temperature for three months and in some cases even six months after preparation. It is possible that the temperature of storage is a deciding factor on the stability, and this is being investigated further. With a reliable and stable substrate slide the L.E. cell test is now very simple to perform, and the finger-prick method should soon find its place in many laboratories.

In 10 S.L.E. patients tested repeatedly by us, moderate numbers or numerous L.E. cells were always found by the finger-prick blood method, using various substrates from lymphatic leukaemia blood. This occurred prior to and during treatment. Following the initiation of treatment with cortisone, symptoms were soon relieved, but in most cases a much longer time elapsed before the L.E. cells in the test diminished.

The finding of an occasional cell resembling the L.E. cell in some normal subjects and in few cases of rheumatoid arthritis requires explanation. Fortunately the finding differs from that in S.L.E., where L.E. cells are found in large numbers by the finger-prick blood method. The dried lymphatic leukaemia serum which remains on the lymphocytes of the substrate may have L.E.-cell-forming properties in minimal amount. Another hypothesis is that minimal amounts of L.E. cell factor are occasionally found in human plasma.

Summary

A method for producing L.E. cells from finger-prick blood is described in which the substrate recommended is obtained from lymphocytes of cases of chronic lymphatic leukaemia.

The method compares favourably with a reliable venous blood method, and has the added advantage of simplicity and ease of microscopical examination.

The substrate slides prepared from lymphocytes appear to be stable for at least three months.

In patients suffering from systemic lupus erythematosus numerous L.E. cells are found *consistently* by the finger-prick method prior to and during treatment.

In some normal subjects, and in a few cases of rheumatoid arthritis, an occasional cell resembling the L.E. cell may be found, but the finding is *not consistent*.

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